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(71) Applicant: **Becton Dickinson and Company**
One Becton Drive
Franklin Lakes, New Jersey 07417-1880 (US)

(72) Inventor: **Fraiser, Melinda S.**
104 East Maynard Avenue
Durham, North Carolina 27704 (JP)
Inventor: **Walker, George Terrance**
209 Mt. Bolus Road
Chapel Hill, North Carolina 27514 (JP)
Inventor: **Schram, James L.**
102 Dwelling Place
Knightdale, North Carolina 27545 (US)

(74) Representative: **Rambelli, Paolo et al**
c/o JACOBACCI & PERANI S.p.A.
Corso Regio Parco, 27
I-10152 Torino (IT)

(54) Decontamination of nucleic acid amplification reactions.

(57) Methods for inactivating contaminating amplicons in isothermal nucleic acid amplification reactions such as SDA, Qβ and 3SR. dU is incorporated into the amplicons produced by amplification in the place of thymine (T). If these amplicons contaminate a subsequent amplification reaction, they may be inactivated as templates (i.e., rendered unamplifiable) by treatment with UDG. As isothermal amplification does not involve elevated temperatures, the UDG may be inactivated during the subsequent amplification of specific target sequences by inclusion of the UDG inhibitor protein Ugi. Incorporation of dU has unexpectedly been found to enhance the amplification power of SDA as compared to conventional SDA reactions. The methods may also be used to detect UDG activity in reagents or samples.

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FIELD OF THE INVENTION

The present invention relates to nucleic acid amplification and in particular to inactivation of amplicons from a previous amplification reaction which may contaminate a subsequent amplification reaction.

BACKGROUND OF THE INVENTION

Nucleic acid amplification reactions are processes by which specific nucleic acid sequences are amplified. They have become powerful tools in nucleic acid analysis and preparation. Several nucleic acid amplification methods are known. These include the polymerase chain reaction (PCR), self-sustained sequence replication (3SR), the ligase chain reaction (LCR), Qβ replicase amplification and strand displacement amplification (SDA). Unfortunately, the powerful ability of these nucleic acid amplification methods to amplify minute quantities of a target sequence also make them susceptible to contamination by target sequences (amplicons) which may be carried over from previous amplification reactions in reagents, pipetting devices and laboratory surfaces. These contaminating products of previous amplifications may themselves be amplified in a subsequent amplification reaction. Even a few molecules of a contaminating target sequence may be readily amplified and detected, resulting in falsely positive results.

A recently developed method for inactivating contaminating amplicons in PCR involves incorporation of the nucleotide deoxyuridine triphosphate (dUTP) into amplified nucleic acid sequences in place of thymidine triphosphate (TTP). As deoxyuridine (dU) is not normally found in naturally-occurring DNA, this nucleotide serves to distinguish previously produced amplicons from new target sequences which have not yet been amplified. The uracil-containing DNAs, representing previously amplified contaminating sequences, are then treated with the enzyme uracil DNA glycosylase (UDG; also known as uracil N-glycosylase or UNG). In nature, uracil DNA glycosylase excises uracil bases from DNA which can arise as a result of either misincorporation by DNA polymerase or deamination of cytosine. For decontamination of PCR amplifications, UDG removes the intentionally incorporated uracil in amplified nucleic acid. Uracil is removed without destruction of the sugar-phosphodiester backbone, thereby producing an abasic site in the DNA. These abasic sites are susceptible to hydrolysis by heat or alkali, a process which fragments the uracil-containing DNA and renders it unamplifiable in subsequent PCR.

As employed to decontaminate PCR, a sample is treated with UDG prior to PCR amplification and the enzyme is inactivated prior to beginning the amplification reaction. This prevents removal of uracil residues from newly generated amplicons. PCR involves cycling between elevated and reduced temperatures. UDG is therefore inactivated after the decontamination treatment by incubation at high temperatures (70-80 • C), a process which is compatible with the PCR. UDG is substantially inactive at the elevated temperatures used for the PCR amplification reactions themselves. However, it has been shown that upon return of the PCR sample to 4 • -25 • C after amplification, sufficient UDG activity is still present to degrade dU-PCR amplification products. It has therefore been recommended that PCR reactions be maintained at elevated temperatures after UDG treatment (Rashtchian, A., Hartley, J.L. and Thornton, C.G., Biotechniques, volume 13,No. 2, page 180). To address the problem of residual UDG activity after heat inactivation, WO 92/01814 describes a thermolabile UDG enzyme. In a further attempt to control residual UDG activity still present after heat inactivation, Rashtchian, et al. have added a protein inhibitor of UDG (Ugi - uracil DNA glycosylase inhibitor) to PCR after heat inactivation of UDG. Ugi is a product of the bacteriophage PBS2 and inhibits host UDG to protect the phage genome during infection, as the phage substitutes dU for T during replication of its genome (Mosbaugh, D.W. and Wang, Z., Journal of Bacteriology, volume 170, No. 3 p.1082). Prior to the present invention, however, there has been no report suggesting the use of Ugi alone to inactivate UDG in the context of decontamination of nucleic acid amplification reactions.

In contrast to the PCR, several nucleic acid amplification methods are isothermal. That is, they do not involve the high/low temperature cycling of the PCR. Examples of isothermal amplification protocols are self-sustained sequence replication (3SR; J. C. Guatelli, et al. *PNAS* 87:1874-1878 (1990), Qβ replicase (P. M. Lizardi, et al. *Bio/Technology* 6:1197-1202 (1988), and strand displacement amplification (SDA; G. T. Walker, et al. *PNAS* 89:392-396 (1992); G. T. Walker, et al. *Nuc. Acids Res.* 20:1691-1696 (1992)). Such isothermal amplification protocols present a particular problem for decontamination, as high temperature steps for inactivation of UDG may not be compatible with the reduced temperature and isothermal nature of the reaction. The SDA amplification protocol is particularly unusual in that it uses both a restriction enzyme and a polymerase to amplify DNA. DNA may be amplified by a factor of 10^8 using this method. The power of the SDA system necessitates the development of a technique to insure that previously amplified material (amplicons) do not inadvertently contaminate fresh reactions. Such contamination may create falsely positive samples. The restriction enzyme used in SDA, HincII, recognizes a specific six base pair

recognition sequence. SDA requires the incorporation of an α-thio derivative of deoxyadenine (dA_s) into the recognition site of HincII by the polymerase in lieu of the naturally occurring dA. The mechanism of SDA is such that the SDA primers form one strand of the restriction site and the polymerase extends the primer to complete the other strand of the site using dA_sTP. The dA_s moiety 3' to the cut site inhibits the restriction of the modified strand. However, it does not inhibit the restriction of the unmodified strand donated by the primer.

Isothermal amplification reactions do not involve elevated temperatures as the PCR does, and it was therefore unknown prior to the present invention whether inclusion of an inhibitor of UDG alone (rather than in conjunction with heat inactivation) would be sufficient to prevent removal of uracil from the desired amplification products. Also, as the literature relating to UDG in PCR emphasizes the role of fragmentation of the abasic nucleic acids in amplicon inactivation (usually by heat), it was not previously known if removal of uracil alone would be sufficient to inactivate contaminating amplicons as templates for further amplification.

In addition to its isothermal nature, SDA differs from the PCR in several other important respects, all of which could have significant effects on the use of UDG for inactivation of contaminating amplicons. First, SDA requires nicking of the DNA by a restriction enzyme, and it has been shown that incorporation of uracil into restriction enzyme recognition sites in some cases prevents restriction. SDA also requires enzymatic displacement of the extended amplification product from the template strand by the polymerase. It was therefore previously not known whether inclusion of uracil in the HincII restriction site and in the amplification product would 1) prevent nicking by HincII (especially as uracil would be base paired with dA_s, and/or 2) prevent normal strand displacement due to the presence of uracil or uracil base-paired with α-thio-A. It was not known whether the polymerase could successfully simultaneously incorporate both unconventional nucleotides, i.e., dUTP and dA_sTP into the amplification products. The SDA KPO_4 buffer system is unique in amplification reactions (PCR uses a Tris buffer). It was unknown whether or not UDG and Ugi would be active in a KPO_4 buffer system. Applicants have unexpectedly found that incorporation of dU into the HincII restriction site does not significantly inhibit nicking by HincII, i.e., the strand without dA_s is still nicked effectively. Further, Applicants have found that incorporation of dU does not significantly interfere with the other enzymatic reactions occurring in SDA, KPO_4 buffer isi compatible with UDG and Ugi activity, and if desired $MgCl_2$ can be eliminated from the reaction.

dU may therefore be incorporated into isothermally-amplified DNA without inhibition of the amplification reaction. The uracil-modified nucleic acids can also be specifically recognized and inactivated by treatment with UDG. Therefore, if dU is incorporated into isothermally-amplified DNA, any subsequent reactions can first be treated with UDG, and any dU containing DNA from previously amplified reactions can be rendered unamplifiable. The target DNA to be amplified will not contain the dU and will not be affected by the UDG treatment. In addition, Applicants have unexpectedly found that UDG can be sufficiently inhibited by Ugi alone prior to amplification of the target, without the heat treatment taught in the prior art. Ugi is therefore useful in isothermal amplification reactions as a means for preventing UDG attack on new amplification products. Ugi may simply be added along with amplification enzymes to begin amplification after decontamination with UDG. These two discoveries have allowed the development of the present UDG/Ugi decontamination method for isothermal nucleic acid amplification reactions.

SUMMARY OF THE INVENTION

The present invention provides methods for inactivating contaminating amplicons in isothermal nucleic acid amplification reactions such as SDA and 3SR. dU is incorporated into the amplicons produced by amplification in the place of thymine (T). If these amplicons contaminate a subsequent amplification reaction, they may be inactivated as templates (i.e., rendered unamplifiable) by treatment with UDG. As isothermal amplification does not involve elevated temperatures, the UDG may be inactivated during the subsequent amplification of specific target sequences by inclusion of the UDG inhibitor protein Ugi.

The incorporation of the dU residues has unexpectedly been found to enhance the amplification power of SDA as compared to conventional SDA reactions. This enhancement is observed when dU-containing nucleic acids are amplified by SDA (without decontamination by addition of UDG). While not wishing to be bound by any particular theory of how the invention works, it is possible that this enhancement is the result of lower melting temperatures when DNA contains dU. These lower DNA melting temperatures may provide greater primer hybridization specificity and may also enhance strand displacement by the polymerase.

DESCRIPTION OF THE DRAWINGS

Fig. 1 is an autoradiograph showing the results of Example 1.

Fig. 2 is an autoradiograph showing the results of Example 2.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides methods for preventing amplicons generated from a prior nucleic acid amplification reaction from serving as templates in a subsequent isothermal amplification reaction. The methods include introduction of an unconventional nucleotide into the amplified target sequence and treatment of subsequent samples for isothermal amplification to specifically remove the unconventional nucleotide prior to amplification of nucleic acids in the sample. Removal of the unconventional nucleotide from the previously generated nucleic acids containing it renders the previously-generated nucleic acid unsuitable for further amplification in the subsequent amplification reaction.

"Unconventional" nucleotides are nucleotides which are not naturally occurring in a particular nucleic acid. Unconventional nucleotides may be naturally occurring nucleotides (e.g., hypoxanthine) or they may be chemically modified derivatives or analogs of conventional nucleotides (e.g., N-7-methylguanine, deoxyuridine and deoxy-3'-methyladenosine). For example, uracil is a naturally occurring and conventional nucleotide in RNA but it is unconventional in DNA. The selected unconventional nucleotide should not inhibit the amplification reaction or subsequent manipulations of the amplified target sequence, i.e., it should not interfere with polymerase, hybridization, etc. Uracil is the preferred unconventional nucleotide for incorporation into DNA according to the methods of the present invention. Uracil is preferably incorporated as 2'-deoxyuridine 5'-triphosphate (dUTP) and may be included in the DNA synthesis reaction during which a target sequence is amplified and/or during synthesis of the primers. Preferably, dUTP is used during amplification (DNA synthesis) to fully or partially replace TTP. Most preferably, dUTP fully replaces TTP in the amplification reaction and is included at a slightly higher concentration than the other nucleotides to drive the reaction for maximum substitution (e.g., 0.5 mM dUTP and 0.2 mM of each of the other dNTPs)-.Longer targets will be more fully dU-substituted than short targets for any given concentration of dUTP, and the concentration of dUTP may be adjusted accordingly depending on target length. In general, each dNTP will be present at 0.1 mM - 1 mM in the amplification reaction. $MgCl_2$ is optional during UDG treatment and if included may be present at about 0.5-10 mM.

dUTP may be substituted fully or partially for TTP in any isothermal amplification reaction as long as it does not adversely affect the amplification. The appropriate amount of dUTP to be included in each amplification system to produce full dU-substitution of the amplification product may be determined experimentally. The amplification is thereafter performed according to known protocols. The preferred isothermal amplification protocol for use in the present invention is SDA.

In general, all amplification reactions in a laboratory will be performed with incorporation of dUTP so that all subsequent amplifications can be decontaminated prior to amplification. To decontaminate a sample prior to amplification, 0.1-10 units of UDG, preferably 1-2 units of UDG, are added to the sample and non-enzymatic amplification reagents (including dUTP) for 5-30 min. at 25-45°C, preferably about 41°C. Following incubation with UDG, the remaining enzymatic components of the amplification are added with about 0.1-50 units of Ugi, preferably 1-4 units of Ugi, to begin the amplification reaction. The ratio of UDG:Ugi should be at least 1:1 or greater, preferably about 4:1. The appropriate amount of Ugi may easily be determined empirically. Addition of Ugi alone is sufficient to inactivate UDG in the reaction and prevent removal of uracil residues from the newly synthesized amplicons.

Amplified target sequences (amplicons) may then be detected using methods known in the art. They may be identified by a characteristic size, for example by gel electrophoresis, or they may be visualized by hybridization to oligonucleotide probes tagged with a detectable label. A preferred method for detecting amplicons is the primer extension method described by Walker, et al. (*Nuc. Acid Res.*, supra), in which a ^{32}-P labeled primer is specifically hybridized to the amplicon and extended with polymerase. An extended primer of the predicted size is then visualized by autoradiography alter gel electrophoresis of the extension products.

UDG is found in many cells, and may contaminate reagents used in nucleic acid laboratory protocols (e.g., restriction enzymes, polymerases, ligases, etc.) The present invention also provides a method for assaying samples and reagents for UDG activity. Such an assay is useful for identifying sources of UDG contamination which may attack uracil-containing DNA. To assay for UDG activity in a sample or a reagent according to the invention, known uracil-containing target nucleic acids are added to the sample or reagent to be tested. The sample or reagent is incubated for a sufficient period of time to allow any contaminating

UDG to remove uracil from the target nucleic acids. Ugi is added and the target nucleic acids are amplified as described above. The amplification products, if any are then detected. If UDG is present in the sample or reagent, no amplification products or a reduced amount of amplification products will be detected. If no UDG is present, amplification of the target nucleic acids will proceed normally.

EXAMPLE 1

This experiment examined the effect of UDG treatment on the amplification of samples containing various amounts (200-50 genomes) of *Mycobacterium tuberculosis* DNA and dU-containing amplicons. dU containing amplicons were produced by SDA of a sample containing approximately $1x10^4$ *Mycobacterium tuberculosis* genomes as described below. The method was generally as described by Walker, et al. *Nuc. Acids Res., supra*, substituting 0.5 mM dUTP for TTP. The remaining dNTPs were included at 0.2 mM. The SDA reaction was run at 41 °C. After amplification, it was estimated that the reaction contained approximately $5.1x10^{11}$ dU-containing amplicons/µl. This preparation was used as the stock source of contaminating amplicons for spiking into new samples, and was diluted as necessary to produce the required number of molecules for each sample.

Mycobacterium tuberculosis (Mtb) DNA and/or 10^5 dU amplicons were contained in 42 µl of reaction buffer as described in Table 1. Reactions were grouped in fours to examine the effect of UDG treatment on the amplicons in the presence or absence of various amounts of genomic Mtb DNA. If the UDG treatment was unsuccessful or only partially successful in eliminating the amplicons, amplification products would be detected in the first sample of each group (samples 1,5,9 and 13). Reaction buffer contained K_iPO_4, bovine serum albumin, four primers (SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4), glycerol, dA_sTP, dCTP and dGTP as previously described (Walker, et al. *Nuc. Acids Res., supra*), but dUTP was substituted for TTP as described above. The samples were denatured at 98°C for 3 minutes, cooled to 42°C and 2 µl of 0.5 unit/µl UDG was added. In the control samples, 2 µl of 25% glycerol was added. All samples were incubated at 42°C for 30 minutes and then the temperature was adjusted to 41°C. SDA amplification was begun by adding 6 µl of an enzyme mix containing $MgCl_2$, exonuclease free Klenow, Hinc II, and 2 or 4 units of the UDG inhibitor (Ugi). The amplification reaction was incubated at 41°C for 2 hours and terminated by heating at 72°C for 2 minutes.

The final concentration of the reaction components after the addition of the enzyme mix was as follows: 50 mM K_iPO_4 pH 7.5; 0.2 mM each dA_sTP, dCTP, dGTP; 0.5 mM dUTP; 0.5 µ M primers 1 and 2 (SEQ ID NO:1 and SEQ ID NO:2); 0.05 uM primers 3 and 4 (SEQ ID NO:3 and SEQ ID NO:4); 0.1 ug/ul bovine serum albumin; 14% glycerol; 1 unit UDG; 2 or 4 units inhibitor; 1 unit exonuclease free Klenow; 150 units Hinc II; 50 ng Human DNA (diluent for Mtb, amplicon DNA)

The products of the UDG/SDA reaction were detected by extension of a ^{32}P-labelled probe (SEQ ID NO:5) and gel electrophoresis analysis as previously described (Walker, et al. *Nuc. Acids Res., supra*). The amplification products were detected as two bands on the autoradiograph corresponding to 35 bases and 56 bases.

Table 1

#	+/-amplicons	Mtb genomes	1U UDG	Ugi
1	$+10^5$	0	yes	2 U
2	$+10^5$	200 genomes	yes	2 U
3	0	200 genomes	yes	2 U
4	0	0	yes	2 U
5	$+10^5$	0	yes	2 U
6	$+10^5$	100 genomes	yes	2 U
7	0	100genomes	yes	2 U
8	0	0	yes	2 U
9	$+10^5$	0	yes	2 U
10	$+10^5$	50 genomes	yes	2 U
11	0	50 genomes	yes	2 U
12	0	0	yes	2 U
13	$+10^5$	0	yes	4 U
14	$+10^5$	50 genomes	yes	4 U
15	0	50 genomes	yes	4 U
16	0	0	yes	4 U
17	$+10^5$	0	no	2 U
18	0	200 genomes	no	2 U
19	0	100 genomes	no	2 U
20	0	50 genomes	no	2 U
21	0	0	no	2 U

The results of this experiment are shown in Fig. 1. No amplification products were detected in samples 1, 5, 9 and 13, indicating successful decontamination of the samples. Sample 17 was a no-UDG control and the amplification products detected indicated that 10^5 contaminating amplicons can be detectably amplified by SDA in the absence of UDG decontamination. Comparing sample 17 to samples 1, 5,9 and 13 provided a measure of the number of amplicons that can be eliminated by the UDG treatment. The second sample in each group of four (samples 2, 6, 10 and 14) provided a measure of the effect of UDG treatment on the amplification of genomic DNA, i.e., genomic DNA was successfully amplified with only a slight loss in signal (compare samples 2, 6, 10 and 14 with the appropriate control of genomic DNA amplified without UDG treatment - samples 18, 19 and 20). The third sample in each group was a control which measured the effect of UDG treatment in the absence of amplicons on the amplification of genomic DNA. That is, if all of the amplicons in samples 2, 6, 10 and 14 were eliminated by the UDG treatment, the amount of SDA amplification product for samples 2 and 3; 6 and 7; 10 and 11; and 14 and 15 (the second and third samples in each set) should be equivalent. The amounts of amplification products produced in these sample pairs were equivalent, demonstrating successful elimination of the contaminating amplicons. The fourth sample in each set was also a control which monitored background (inadvertent) amplicon contamination that might be added to the SDA reaction with the SDA reagents. These samples contained only 50 ng of human DNA (used as a diluent) which is not specifically amplified by the SDA primers. Samples 4, 8, 12 and 16 were completely free of amplification products. However, sample 21, which received no UDG treatment, showed a weak amplification product signal. The presence of amplification product in sample 21 indicated that the buffer mix had been accidentally contaminated by a low level of amplicons (less than the equivalent of 50 genomes). Comparing this lane to the fourth lane in the UDG treated sets (samples 4, 8, 12 and 16) demonstrated that the background amplicons were also eliminated from the samples by UDG.

These experiments demonstrated that as many as 10^5 dU-containing contaminant amplicons can be eliminated, while still allowing successful amplification of as few as 50 M.tb genomes.

EXAMPLE 2

This experiment examined the effect of $MgCl_2$ on the UDG treatment of 10^5 contaminating amplicons and the effect of time on the treatment. The dU containing amplicons were those generated by SDA amplification in Example 1. After amplification it was estimated that the reaction contained $5.1x10^{11}$ amplicons/µl. The amplicons were diluted as needed to provide the $1x10^5$ dU amplicons used to evaluate UDG decontamination.

Reaction mixes (42 µl) contained K_iPO_4 pH 7.5, bovine serum albumin, dUTP, dA_sTP, dCTP, dGTP, primers 1-4 (SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4), and glycerol. In addition, this mix contained +/- $MgCl_2$, +/- dU and M.tb genomic DNA as indicated in Table 2. The samples were heated at 98°C for 3 minutes to denature the DNA and then cooled to 42°C. Two microliters of 0.5 units/µl UDG was added as indicated in Table 2 and allowed to incubate for 5 minutes, 15 minutes or 30 minutes. Enzyme mixtures were prepared containing +/- $MgCl_2$, Hinc II, exonuclease free Klenow and Ugi. Six microliters of enzyme mixture was added to the samples after UDG treatment to begin the SDA amplification reaction. Samples were incubated at 41°C for 2 hours and amplification was terminated by heating for 2 minutes at 72°C. The final concentration of all the reaction components alter the addition of the enzyme mix was as follows: 50 mM K_iPO_4 pH 7.5; 0.2 mM each dA_sTP, dCTP, dGTP; 0.5 mM dUTP; 0.5 µM primers 1 and 2 (seq. I.D. No. 1&2); 0.05 µM primers 3 and 4 (SEQ ID NO:3 and SEQ ID NO:4); 0.1 µg/µl bovine serum albumin; 7 mM $MgCl_2$; 14% glycerol; 1 unit UDG; 2 units Ugi; 1 unit exonuclease free Klenow; 150 units Hinc II; 50 ng human DNA (diluent for amplicons and Mtb DNA).

The products of the UDG/SDA reaction were detected by extension of a ^{32}P-labelled probe (SEQ ID NO:5) and gel electrophoresis analysis as in Example 1. The SDA amplification products were detected as two bands on an autoradiograph, corresponding to 35 bases and 56 bases.

Table 2

#	+/-amplicons	+/-50 genomes	UDG time	+/- $MgCl_2$ during treatment UDG
1	$+10^5$	0	5 min	+ $MgCl_2$
2	$+10^5$	+50 genomes	5 min	+ $MgCl_2$
3	0	+50 genomes	5 min	+ $MgCl_2$
4	$+10^5$	0	5 min	-$MgCl_2$
5	$+10^5$	+50 genomes	5 min	-$MgCl_2$
6	0	+50 genomes	5 min	-$MgCl_2$
7	$+10^5$	0	15 min	+ $MgCl_2$
8	$+10^5$	+50 genomes	15 min	+ $MgCl_2$
9	0	+50 genomes	15 min	+ $MgCl_2$
10	$+10^5$	0	15 min	-$MgCl_2$
11	$+10^5$	+50 genomes	15 min	-$MgCl_2$
12	0	+50 genomes	15 min	-$MgCl_2$
13	$+10^5$	0	30 min	+ $MgCl_2$
14	$+10^5$	+50 genomes	30 min	+ $MgCl_2$
15	0	+50 genomes	30 min	+ $MgCl_2$
16	$+10^5$	0	30 min	-$MgCl_2$
17	$+10^5$	+50 genomes	30 min	-$MgCl_2$
18	0	+50 genomes	30 min	-$MgCl_2$
19	0	+50 genomes	no UDG	+ $MgCl_2$
20	0	+50 genomes	no UDG	-$MgCl_2$

The results are shown in Fig. 2. The reactions were grouped in sets of three to examine the effect of $MgCl_2$ and time on the ability of the UDG enzyme to effectively eliminate 10^5 dU amplicons. If the UDG treatment was unsuccessful or only partially successful the first sample in each set (samples 1, 4, 7, 10, 13 and 16) would contain amplification products. The absence of amplification products in samples 1, 4, 7, 10, 13 and 16 indicated that the UDG enzyme was able to eliminate 10^5 contaminating dU amplicons under all of the conditions tested. As was also shown in Example 1, the presence of amplification products in the second sample of each set (samples 2, 5, 8, 11, 14 and 17) demonstrated the ability of SDA to amplify genomic DNA in the presence of UDG treated amplicons even in the absence of a heating step between the UDG treatment and the SDA reaction. The prior art relating to the PCR has taught that the heating step was necessary not only to inactivate the UDG enzyme but to cause the abasic, UDG treated DNA to be fractionated into smaller, non-amplifiable segments. Applicants have determined that this heating step is not necessary and have discovered that Ugi alone is sufficient to inactivate UDG. This indicates that the abasic but intact DNA is not amplified under the conditions tested. The third sample in each set (samples 3, 6, 9, 12, 15 and 18) was a control to measure the effect of the UDG treatment on the amplification of genomic DNA in the absence of contaminating dU amplicons. Comparison of the amplification products of the second and third samples in each set demonstrated equivalent signals. There is therefore no difference between the amplification of genomic DNA in the presence or absence of UDG treated amplicons. The last two samples were controls (samples 19 and 20) to measure the amplification of 50 genomes of *Mycobacterium tuberculosis* without UDG treatment. The signal from the untreated sample was slightly darker than that of the UDG-treated sample. This difference suggests that SDA amplification is slightly more efficient in the absence of UDG treatment. This example demonstrated that the presence or absence of $MgCl_2$ during UDG treatment has no significant effect on the elimination of 10^5 contaminating dU amplicons. In addition, a 5 minute incubation was sufficient to eliminate 10^5 dU amplicons independent of the presence of $MgCl_2$.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: BECTON, DICKINSON AND COMPANY
(A) NAME: BECTON, DICKINSON AND COMPANY
(B) STREET: 1 Becton Drive
(C) CITY: Franklin Lakes
(D) STATE: NJ
(E) COUNTRY: US
(F) ZIP: 07417

(ii) TITLE OF INVENTION: DECONTAMINATION OF NUCLEIC ACID AMPLIFICATION REACTIONS

(iii) NUMBER OF SEQUENCES: 5

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(v) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TTGAATAGTC GGTTACTTGT TGACGGCGTA CTCGACC 37

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TTGAAGTAAC CGACTATTGT TGACACTGAG ATCCCCT 37

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TGGACCCGCC AAC 13

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CGCTGAACCG GAT 13

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CGTTATCCAC CATAC 15

Claims

1. A method for preventing amplification of contaminating amplicons generated in a prior isothermal amplification reaction during subsequent isothermal amplification of a sample, the method comprising the steps of:
   a) incorporating uracil into the amplicons during the prior isothermal amplification reaction;
   b) prior to the subsequent isothermal amplification, treating the sample with a sufficient amount of uracil DNA glycosylase (UDG) to render the contaminating amplicons unamplifiable, and;
   c) amplifying the treated sample in the presence of a sufficient amount of uracil-DNA glycosylase inhibitor (Ugi) to inactivate the UDG.

2. The method according to Claim 1 wherein the prior and subsequent amplifications are by Strand Displacement Amplification.

3. The method according to Claim 2 wherein about 0.1-1 mM dUTP is included in the prior amplification reaction.

4. The method according to Claim 3 wherein about 0.5 mM dUTP is included in the prior amplification reaction.

5. The method according to Claim 3 wherein about 0.1-1 mM dUTP is included in the subsequent amplification reaction.

6. The method according to Claim 5 wherein about 0.5 mM dUTP is included in the subsequent amplification reaction.

7. The method according to Claim 2 wherein the sample is treated with about 0.1-10 units of UDG and the treated sample is amplified in the presence of about 0.1-50 units of Ugi.

8. The method according to Claim 7 wherein the sample is treated with about 1-2 units of UDG and the treated sample is amplified in the presence of about 1-4 units of Ugi.

9. A method for detecting uracil DNA glycosylase (UDG) activity in a sample comprising:
   a) adding uracil-containing nucleic acid to the sample;
   b) isothermally amplifying the uracil-containing nucleic acid, and;
   c) detecting the amplified nucleic acid as an indication of UDG activity.

10. The method according to Claim 9 wherein the nucleic acid is amplified by Strand Displacement Amplification.

FIG-1

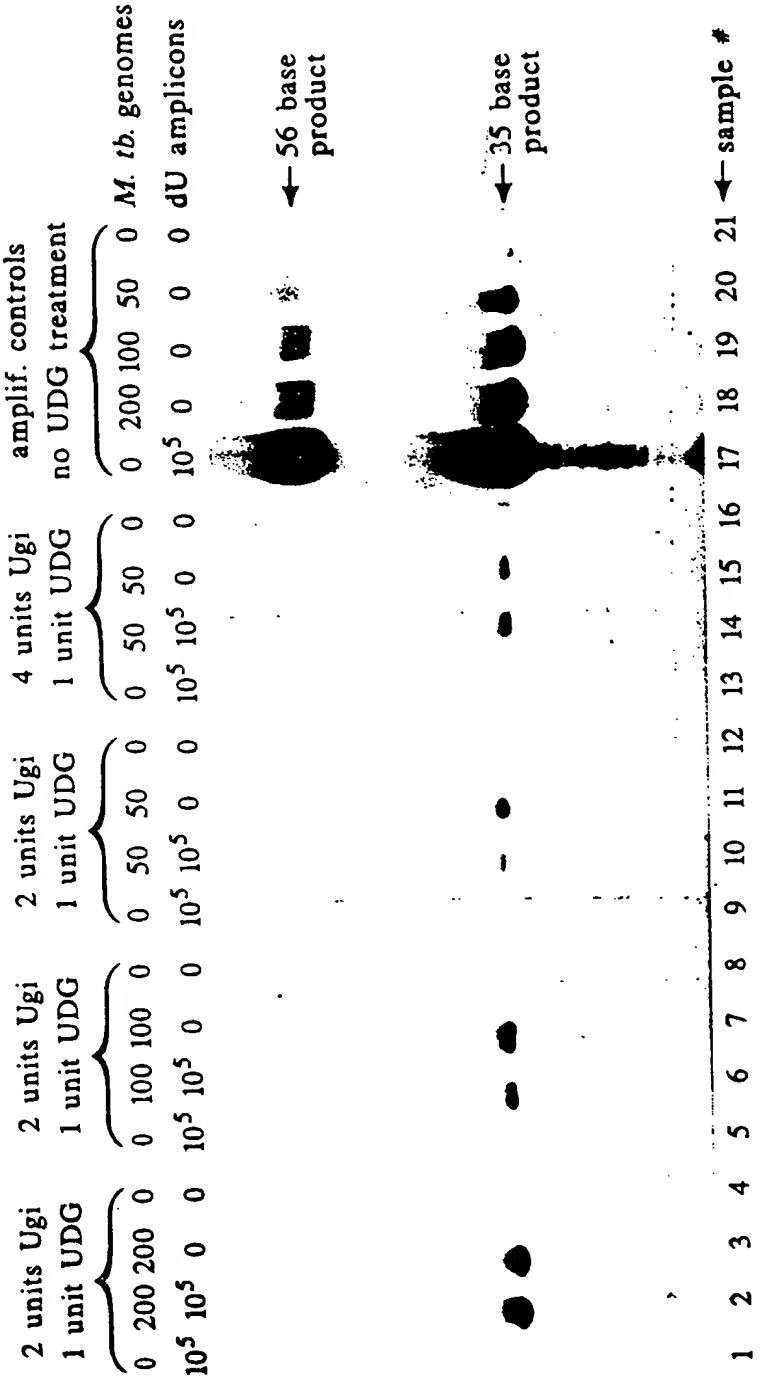

FIG-2